that small changes in physical parameters can significantly alter this response. Supported by NSF grant MCB- 115803 and by NIH postdoctoral fellowship GM087099 to N.V.N.

1742-Plat
Effects of Crowding, Osmolytes, Temperature and Pressure on the Interaction Potential of Dense Protein Solutions
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We studied the effect of pressure on the structure and intermolecular interactions of dense lysozyme solutions in various cosolvent mixtures and upon addition of various Hofmeister anions using small-angle X-ray scattering in combination with liquid-state theoretical approaches [1-3]. Supplementary thermodynamic information was obtained by employing calorimetric techniques, densitometry and ultrasound velocimetry. We show that the particular structural properties of water and specific ion effects play a crucial major role in protein stabilisation, notably under high hydrostatic pressure conditions. Also the effect of confinement on the solvational properties and intermolecular interaction of proteins was studied, including the effects of self-crowding and macromolecular crowders on the temperature-pressure stability diagram of proteins [4]. We also discuss the effect of pressure on the second virial coefficient and how pressure can be used to control and fine-tune protein crystallization. Moreover, we present results on the phase behavior of dense lysozyme solutions in the liquid-liquid phase separation region. A re-entrant liquid-liquid phase separation region has been discovered at elevated pressures, which originates in the pressure dependence of the solvent-mediated protein-protein interactions [3].
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1743-Plat
A Multiscale Model for pH-Dependent Folding and Binding of a Conditionally Disordered Chaperone
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The bacterial acid stress-sensing chaperone HdeA loses structure to gain function. As enteropathogenic E. coli pass through the severely acidic environment of the mammalian stomach, HdeA transitions from an inactive, folded dimer to chaperone-active, unfolded monomers to protect against the acid-induced aggregation of periplasmic proteins. Toward achieving an atomic-level mechanistic understanding of the acid stress response of HdeA, we develop a multiscale modeling approach to capture its pH-dependent thermodynamics. Our approach utilizes pKa calculations from all-atom constant pH molecular dynamics simulations to alter the coarse-grained model for representing different pH environments. Changes in the thermodynamics of binding as a function of pH are explored using the efficient ''Hamiltonian mapping'' reweighting formalism. We propose new features of the pH-sensing mechanism of HdeA that can be directly tested by experiment. Namely, our model predicts that HdeA is maximally stable under mildly acidic conditions and that a partially unfolded dimeric intermediate may contribute to substrate binding. Our multiscale approach is general such that it can be applied toward understanding pH-dependent functional transitions in other systems and sets a foundation from which to construct models of HdeA-substrate interaction.

1744-Plat
Structural Origin of Landscape Roughness in Protein Folding from Single-Molecule FRET and All-Atom Molecular Dynamics Simulations
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Folding of most single-domain proteins has been successfully described by diffusion on a one-dimensional (1D) free energy surface. Although the 1D surface is smooth, there are many local minima in the underlying energy landscape, giving rise to landscape ''roughness''. According to Kramers' reaction-rate theory, roughness slows folding kinetics by reducing the diffusion coefficient at the top of the free energy barrier that separates folded and unfolded states. By measuring the transition-path time (t_{TP}) from a maximum likelihood analysis of photon trajectories in single molecule FRET experiments, we have recently shown that the Kramers diffusion coefficient for a designed α-helical protein, α_3D, is markedly reduced (Chung and Eaton, *Nature*,2013). To discover the structural origin of this slow diffusion, we have combined additional single-molecule FRET measurements with all-atom molecular dynamics (MD) calculations. α_3D contains 12 negatively-charged and 10 positively-charged side-chains. Analysis of the transition paths in the MD simulations shows that many non-native salt-bridges form during the folding transition path, suggesting them as the structural origin of long t_{TP} . To test this idea, we lowered the pH to neutralize the carboxylates and eliminate salt-bridges, which increased the folding rate by about 10-fold and significantly reduced t_{TP}. Although it was only possible to determine an upper bound for t_{TP}, even at the highest possible solvent viscosity (15 cP), simulations of photon trajectories suggested that most, if not all, of the increase in folding rate could be accounted for by a decreased t_{TP} and an increased Kramers diffusion coefficient. Neutralizing the carboxylates in MD simulations also increases the folding rate and diffusion coefficient and decreases t_{TP}. These results provide the first quantitative glimpse of the effect of specific intra-molecular interactions on barrier crossing dynamics in protein folding.

1745-Plat
Resolving Cooperative Interactions in Protein Folding
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Understanding the intricate relationships between protein sequence, structure, and stability remains a challenge. One major obstacle in understanding these relationships is the identification of interactions that couple elements of structure; these interactions are not readily apparent from structure. Recently, the application of nearest-neighbor models to repeat protein folding have begun to provide insights into how these systems distribute their energy within, and between units of protein structure1,2,3. Consensus ankyrin and 34-residue cTPR protein (aka c34PR) folding can be described using two terms: the intrinsic energy of individual repeats (ΔGi), and the interfacial energy between adjacent repeats (ΔGi,i+1).
Here, we extend this approach in two ways using two experimental TPR-like systems. First, we dissect the whole-repeat nearest-neighbor model by representing (and resolving) the subunits of the nearest-neighbor model into each of the two helices (''A'' and ''B'') of each repeat. This extended Ising analysis allows us to quantify coupling energies within and between repeats, as well as local stability differences of the A and B repeat helices. Second, we explore a new, longer class of TPR-like proteins with 42 residues per repeat (42PRs, as opposed to 34 residues for the founding TPR sequence motif), to determine how variation in helix length and interface structure affects intrinsic and coupling energy.
By varying the length of constructs in half-repeat increments, we find energetic heterogeneity at both the intrinsic and interfacial level. Although stabilities are more homogeneously distributed in the c34PR series (ΔGAB and ΔGBA as well as ΔGA and ΔGB are nearly isoenergetic), the distribution is more heterogeneous within 42PRs, with a greater energetic separation between intrinsic and interfacial energies. This leads to greater folding cooperativity within the 42PR series.
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1746-Plat
Mapping the Mechanism of Fast Protein Folding with Multiple Probes
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Unlike reactions of small organic molecules, it is not always possible to accurately describe the process of protein folding by a single reaction coordinate. We enhance the structural resolution of microsecond protein folding experiments by introducing new fluorescent inter-helical contact probes into the model protein lambda repressor fragment 6-85. We design two new mutants containing tryptophan and tyrosine residues that interact in the native state. Temperature jump relaxation experiments on these new mutants, in